Title: Effectiveness of Chlorine and Nisin-EDTA Treatments of Whole Melons and Fresh-Cut Pieces for Reducing Native Microflora and Extending Shelf-Life

Author(s): D.O. Ukuku and W.F. Fett

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EFFECTIVENESS OF CHLORINE AND NISIN-EDTA TREATMENTS OF WHOLE MELONS AND FRESH-CUT PIECES FOR REDUCING NATIVE MICROFLORA AND EXTENDING SHELF-LIFE[1]

DIKE O. UKUKU[2] and WILLIAM F. FETT

U.S. Department of Agriculture, Agriculture Research Service
Eastern Regional Research Center
600 East Mermaid Lane
Wyndmoor, PA 19038

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ABSTRACT

Efficacy of nisin-EDTA treatments as a sanitizing treatment for reducing native microflora of whole melons and extending shelf-life of fresh-cut pieces was compared to chlorine treatments. Whole cantaloupe and honeydew melons were washed with water, nisin (10 μg/mL)-EDTA (0.02 M), or 200 ppm chlorine for 5 min at ~ 20C before fresh-cut preparation and storage at 5C for 15 days with periodic microbiological sampling. In addition, some fresh-cut pieces were washed with 10 μg/mL nisin-EDTA or 50 ppm chlorine for 1 min before storage. Changes in appearance, odor, overall acceptability and the shelf-life of the minimally processed fresh-cut melons were investigated. Preliminary studies indicated that water washes, EDTA (0.002 to 0.2 M) or nisin (5 to 10 μg/mL) were not effective in reducing the microflora of whole melon when used individually. Nisin-EDTA and chlorine treatments were significantly (P < 0.05) more effective in reducing native microflora than water washes. Nisin-EDTA treatments were significantly (P < 0.05) more effective than chlorine in reducing populations of yeast and mold and Pseudomonas *spp. on whole melon surfaces but were not as effective as chlorine treatments for reducing aerobic mesophilic bacteria, lactic acid bacteria and total gram-negative bacteria. Microbial contaminants on fresh-cut pieces washed with 50 ppm chlorine or nisin-EDTA were further reduced. However, microbial populations increased throughout refrigerated storage irrespective of treatments. Odor, appearance, and overall acceptability ratings for cantaloupe and honeydew fresh-cut pieces treated with nisin-EDTA or chlorine were not significantly (P > 0.05) different from each*

[1] Mention of brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture over others of similar nature not mentioned.

[2] Corresponding author: D.O. Ukuku, E-mail: dukuku@arserrc.gov.

other throughout the storage period (15 to 21 days). However, both treatments led to significantly (P < 0.05) improved ratings compared to the controls for the fresh-cut pieces at 9 to 12 days of storage and thereafter. The results of this study suggest that treatments with nisin-EDTA before and after fresh-cut processing would improve the quality and extend the shelf-life of fresh-cut melon.

INTRODUCTION

Cantaloupe (*Cucumis melo L. var. cantalupenis* Naud.) and honeydew (*Cucumis melo L. var. inodorus* Naud.) muskmelon are popular fruits in United States and other parts of the world. The surface of cantaloupe muskmelon is covered with a meshwork of raised tissue commonly referred to as the "net" (Webster and Craig 1976) while honeydew muskmelon lacks the "net" tissue. Muskmelon surfaces, like most vegetables, are frequently in contact with the soil (Beuchat 1995) and their surfaces are not free from natural contaminants. There are several reports of enteric disease due to consumption of melons that were contaminated on their surfaces with bacterial human pathogens such as *Salmonella* (CDC 1991; FDA 2000). Therefore, the safety of fresh-cut melons and other produce available in salad-bar operations and supermarkets is a concern (Hurst and Schuler 1992; FDA 2000). The level of sanitation and the microbiological load are of primary importance to the quality, shelf stability and safety of fresh produce (Brackett 1992; Madden 1992; Marston 1995).

Physical and chemical treatments are used in food processing to eliminate or at least reduce the presence of pathogenic and spoilage microorganisms (Wei *et al.* 1985; Ray 1992). Washing is one of the very first processing operations to which a fruit or vegetable is subjected. Chlorination of wash water up to 200 ppm is routinely applied to reduce microbial contamination in produce processing lines (Wei *et al.* 1985). However, the use of chlorine is of concern due to the potential formation of harmful by-products (Richardson *et al.* 1998) and can only achieve approximately 2 to 3 log reductions of native microflora (Ukuku *et al.* 2001). Thus, there is much interest in developing safer and more effective sanitizers.

Nisin is a pentacyclic heterodetic (Gross and Morell 1971; Shiba *et al.* 1991) subtype A lantibiotic peptide synthesized by *Lactococcus lactis* subsp. lactis (Jung 1991a, b). It is an effective inhibitor of gram-positive bacteria (Benkerroum and Sandine 1988; Ukuku and Shelef 1997; Harris *et al.* 1991; Ray 1992) and bacterial spores (Harris *et al.* 1991; Ray 1992). The cytoplasmic membrane appears to be the site of action (Montville and Chen 1998; Bruno *et al.* 1992; Ruhr and Sahl 1985; Okereke and Montville 1992). Nisin has both hydrophobic and hydrophilic regions (Gross and Morell 1971). This structural diversity contributes to nisin's high surface activity and increased capacity for adsorption. In the United States, nisin has received GRAS (generally recognized as safe) status and is approved for use in some processed cheese spreads to prevent the outgrowth of clostridial spores and toxin production (Benkerroum and Sandine 1988). There are several reports that nisin used in combination with a chelating agent exhibits a bactericidal effect towards both gram-positive and gram-negative bacteria (Blackburn *et al.* 1989; Cutter and Siragusa 1995; Stevens *et al.* 1991, 1992a, b). In the current study, the efficacy of nisin treatments in combination with ethylenediaminetetraacetic acid (EDTA) in reducing the native microflora on whole cantaloupe and honeydew melons was compared to that of chlorine treatments. Also, the shelf-life, appearance, odor and overall acceptability of fresh-cut pieces dipped in nisin-EDTA was compared to chlorine-treated fresh-cut pieces during storage at 5C.

MATERIALS AND METHODS

Cantaloupe and Honeydew Melons

Cantaloupes (1651 $\pm$ 54 g) and honeydew (1881 $\pm$ 38 g) melons were purchased from a local wholesale distribution center. Melons were stored at 4C. Before use, melons were unpacked and placed on the laboratory bench for ~18 h to allow them to come to room temperature (~20 C).

Preparation of Wash Solutions

A stock solution of nisin (10^6 I. U., Sigma, St. Louis, MO) was prepared at a concentration of 2500 μg/mL in 0.02 N hydrochloric acid (HCl, pH 2). The stock solution was filter sterilized (0.22 μm, Millipore, Bedford, MA), and stored at -80C until used. A stock solution of 2 M disodium EDTA (Fisher Scientific Co., Pittsburgh, PA) was prepared in deionized distilled water (ddH_2O), autoclaved at 121C for 15 min and then stored at room temperature until used. The test solutions were prepared as follows; (1) disodium EDTA was added to sterile tap water to give final concentrations of 0.002 M, 0.02M or 0.2 M, (2) nisin was prepared by dilution of the stock solution in ddH_2O to give 0, 5 and 10 μg/mL of nisin, and (3) nisin was dissolved in 0.02 M EDTA at a final concentration of 5 and 10 μg/mL. Clorox, a commercial bleach containing 5.25% sodium hypochlorite (NaOCl, Clorox Co., Oakland, CA), was diluted with sterile water to provide a concentration of 200 ppm of available chlorine in the wash solution. The pH for the wash solution was adjusted to 6.0 $\pm$ 0.1 by adding citric acid. Free chlorine in the solution was determined with a chlorine test kit (Hach Co., Ames, IA) that has been approved by the U.S. Environmental Protection Agency.

Washing Treatments

Washing treatments were performed by totally submerging the melons in 3 L sterile tap water with or without EDTA, nisin, a combination of nisin-EDTA or chlorine and manually rotated to assure complete coverage and contact of surfaces with the wash solution for 5 min. Washed melons were placed on crystallizing dishes inside a biosafety cabinet to dry for 1 h.

Preparation of Fresh-Cut Pieces

To prepare fresh-cut pieces, whole cantaloupe or honeydew melons washed with water were cut into four sections using a sterile knife and the rinds carefully removed. The interior flesh was cut into ~3 cm cubes and pieces were washed for 1 min with either sterile tap water, 50 ppm chlorine or 10 μg/mL nisin-EDTA (0.02 M) before placing inside a Stomacher® (Dynatech Laboratories, Alexandria, VA) bag (100 g per bag). Bags containing the pieces were stored at 5 C for up to 20 days.

Microbiological Analyses

A sterilized stainless steel cork-borer was used to cut through the cantaloupe or honeydew whole melon surfaces at random locations to produce rind plugs of 22 mm in diameter with a rind surface area (πr^2) of 3.80 cm^2. Flesh adhering to the rind plugs was trimmed off using a sterilized stainless steel knife. Forty melon rind plugs per whole melon weighing approximately 20 g were blended (Waring commercial blender, Dynamic Corp, New Hartford, CT, speed set at level 5) for 1 min with 80 mL of sterile 0.1% peptone water. Decimal dilutions of the sample were made with 0.1% peptone water, and aliquots (0.1 mL) were plated in duplicate on a range of media. Plate Count Agar (PCA, Difco Becton Dickinson, Sparks, MO) with incubation at 30C for 3 days was used for enumeration of mesophilic aerobes. PCA + crystal-violet (2 mg/mL) with incubation at 30C for 3 days was used for enumerating gram-negative bacteria (Cousin *et al.* 2001). *Pseudomonas* spp. were enumerated by plating 0.1 mL on *Pseudomonas* isolation agar (Difco) with incubation at 27C for 3 days. Lactic acid bacteria were enumerated with de Man, Rogosa and Sharpe agar (MRS, Oxoid, Ogdensburg, NY) with 0.08% sorbic acid as a supplement with incubation at 30C for 3 days (Reuter 1985). Yeast and mold were enumerated according to Norris and Ribbons (1971) using Czapek Malt Agar (CMA, Sigma, St. Louis, MO).

For fresh-cut pieces before or after storage, 200 mL of 0.1% peptone water was added to Stomacher® bags containing fresh-cut pieces (100 g/bag) and the bag contents pummeled for 30 s in a Stomacher® model 400 (Dynatech Laboratories, Alexandria, VA) at medium speed. Before storage at 5C or after

3 days of storage, undiluted samples (0.1 mL) were plated on the different agar media as stated above. For fresh-cut pieces stored for more than 3 days, decimal serial dilutions prepared in 0.1% peptone water were plated on the agar media.

Quality Evaluation

Panelist selection and training and quality evaluation were undertaken as described by O'Connor-Shaw *et al.* (1995), which was a slight modification of a procedure described by Kader *et al.* (1973). Fresh-cut melon pieces were scored for appearance, odor and overall acceptability using a predetermined list of descriptors (Table 1). A panel of five judges was used to evaluate the quality of fresh-cut melons during refrigerated storage (5C) at days 0, 3, 6, 9, 12, 15, 18 and 21. On each day of testing, panelists were presented with freshly cut melon pieces as a reference.

Data Analysis

All experiments were replicated 3 times. Data from each treatment were subjected to the Statistical Analysis System (SAS Institute, Cary, NC) for analysis of variance (ANOVA) and the Bonferroni LSD method (Miller 1981) to determine significant differences between treatments and storage temperatures.

RESULTS

Native Microflora of Whole Melon Rind and Fresh-Cut Pieces

Initial populations of native microflora on the rind of unwashed whole cantaloupes and honeydew melons are shown in Table 2. The surface of cantaloupe melon supported higher populations of all classes of microbes enumerated than the surface of honeydew melon. The predominant class of organisms on cantaloupe and honeydew melon were aerobic mesophilic bacteria followed by lactic acid bacteria, total gram-negative bacteria, yeast and mold and *Pseudomonas* spp. Untreated fresh-cut pieces prepared from water washed cantaloupe melon were also found to have higher microbial populations than similarly prepared fresh-cut pieces of honeydew. Immediately after cutting, the population for total mesophilic aerobic bacteria determined on fresh-cut pieces of cantaloupe and honeydew melon were 3.33 and 2.07 log CFU/g, respectively. Populations of lactic acid bacteria determined on fresh-cut cantaloupe and honeydew melon were 2.08 log CFU/g and below detection, respectively. Gram-negative bacteria, yeast and mold and *Pseudomonas* spp. on fresh-cut cantaloupe and honeydew pieces were below the limit of detection.

TABLE 1.
RATING SCALE FOR APPEARANCE, ODOR AND OVERALL ACCEPTABILITY FOR FRESH-CUT MELONS

Measurement	Score[a]	Quality description
Appearance	1	dislike extremely, very poor, not usable
	3	dislike moderately, poor, excessive defects, limited salability
	5	neither like or dislike, borderline, fair, slightly to moderately objectionable defects, lower limit of appeal
	7	like moderately, good, minor defects, not objectionable
	9	like extremely, excellent, essentially free from defects, fresh-like and typical
Odor		
	1	extremely dislike,
	3	unacceptable, poor, stale, musty and moldy
	5	fairly acceptable,
	7	good, not objectionable, acceptable
	9	excellent, typical, very much acceptable
Overall Acceptability		
	1	dislike, extremely poor
	3	dislike, moderately poor
	5	neither like or dislike, fair, may buy from the store
	7	very good, will definitely buy
	9	extremely good, most definitely buy

[a]Panelist were asked to use intermediate scores where they deemed appropriate.

TABLE 2.
EFFECT OF NISIN-EDTA AND CHLORINE TREATMENTS ON THE NATIVE MICROFLORA OF WHOLE MELONS

Treatment	Survivors ($\log_{10}$ CFU/cm^2)[a]				
	APC[b]	Gram-negative bacteria	LAB[b]	Yeast & Mold	*Pseudomonas* spp.
Cantaloupe					
Control	6.82 ± 0.23[a]	3.10 ± 0.10[a]	4.40 ± 0.15[a]	2.38 ± 0.15[a]	2.04 ± 0.13[a]
Water	6.60 ± 0.14[a]	3.00 ± 0.14[a]	4.32 ± 0.13[a]	2.03 ± 0.12[a]	2.00 ± 0.10[a]
Cl_2 (200 ppm)	3.38 ± 0.15[c]	1.24 ± 0.11[c]	1.34 ± 0.12[b]	0.92 ± 0.10[b]	1.76 ± 0.11[b]
Nisin-EDTA*	4.55 ± 0.14[b]	2.39 ± 0.12[b]	4.17 ± 0.14[a]	0.42 ± 0.12[c]	0.95 ± 0.06[c]
Honeydew					
Control	3.51 ± 0.16[a]	2.30 ± 0.13[a]	2.28 ± 0.14[a]	1.04 ± 0.09[a]	1.30 ± 0.13[a]
Water	3.40 ± 0.14[a]	2.24 ± 0.10[a]	2.32 ± 0.11[a]	0.89 ± 0.04[b]	1.26 ± 0.10[a]
Cl_2 (200 ppm)	1.30 ± 0.15[c]	0.69 ± 0.13[c]	0.49 ± 0.12[c]	0.57 ± 0.10[c]	0.76 ± 0.11[b]
Nisin-EDTA*	2.07 ± 0.10[b]	1.45 ± 0.11[b]	2.07 ± 0.10[a]	0.22 ± 0.12[d]	0.35 ± 0.08[c]

[a] Values represent means for data from three experiments with duplicate determinations per experiment. Means in the same column for each type of melon not followed by the same letter are significantly ($P<0.05$) different.

[b] APC = Aerobic mesophilic bacteria, LAB = Lactic acid bacteria.

* Nisin-EDTA = 10 µg/mL nisin in 0.02 M EDTA

Effect of Washing Treatments on Microflora of Whole Melons

In preliminary experiments, washing whole melons with water, EDTA (0.002 to 0.2 M) and nisin (5 to 10 µg/mL) when tested alone, did not cause any significant ($P>0.05$) reduction in all five categories of native microflora on the melon surfaces (data not shown). Next, a combination of EDTA and nisin were tested as a washing treatment and compared to a chlorine (200 ppm) wash. The combination treatments with nisin (10 µg/mL)-EDTA (0.02 M) were significantly ($P<0.05$) more effective than washing with water alone for reducing aerobic mesophilic bacteria, gram-negative bacteria, *Pseudomonas* spp. and yeast and molds, but not lactic acid bacteria (Table 2). The chlorine wash was significantly ($P<0.05$) more effective than the nisin-EDTA combination treatment against all groups of microbes except *Pseudomonas* spp. and yeast and mold (Table 2). For these two categories of microbes, nisin-EDTA treatment was significantly ($P<0.05$) more effective. Washing with nisin-EDTA reduced the total aerobic mesophilic bacteria on the cantaloupe rinds by 2.27 log

CFU/cm^2 as compared to a 3.44 log reduction for the chlorine treatments. The nisin-EDTA treatment reduced gram-negative and lactic acid bacteria on the cantaloupe rinds by 0.71 log CFU/cm^2 and 0.33 log CFU/cm^2 respectively, while yeast and mold was reduced by 1.96 log CFU/cm^2. Overall, the data indicated that chlorine treatments were more effective in reducing the native microflora on the cantaloupe rind than the nisin-EDTA treatment. Similar results were observed for honeydew melon (Table 2).

Effect of Treatment on Native Microflora on Fresh-Cut Melon During Storage

The effect of washing whole cantaloupe melon with chlorine (200 ppm) or nisin (10 μg/mL)-EDTA (0.02 M) on the native microflora of fresh-cut pieces prepared from the washed melons during refrigerated storage (5C) is shown in Fig. 1. Overall, fresh-cut pieces prepared from whole cantaloupe melons washed with chlorine had the lowest microbial population followed by fresh-cut pieces from nisin-EDTA treated whole melons. Populations of all groups of microorganisms including *Pseudomonas* spp. increased in all samples as storage time increased, regardless of the treatment. Directly treating fresh-cut pieces with chlorine or nisin-EDTA was even more effective in reducing microbial populations during storage than treatments of whole melons alone before fresh-cut preparation. Washing of fresh-cut pieces prepared from water washed whole melon with chlorine (50 ppm) or nisin (10 μg/mL)-EDTA (0.02 M) led to greater control of native microflora and a delay in microbial growth than did washing of fresh-cut cantaloupe pieces with water (Fig. 2) or for unwashed pieces (data not shown). Chlorine treatments were more effective than treatment with nisin-EDTA. All populations for each microbial category on fresh-cut pieces directly treated with chlorine or nisin-EDTA were much lower than the untreated controls. Again, the populations of aerobic mesophilic bacteria (APC), total gram-negative bacteria, lactic acid bacteria, yeast and mold and *Pseudomonas* spp. increased in all samples as storage time increased, regardless of the treatment.

The effect of washing whole honeydew melons with chlorine or nisin-EDTA on the native microflora on fresh-cut pieces during refrigerated storage is shown in Fig. 3. Overall, the results were similar to those found for cantaloupe melons with the chlorine wash being the most effective in reducing microbial populations. The populations of all five categories of microorganisms on fresh-cut honeydew melon increased as storage time increased, regardless of the treatment before fresh-cut preparation. As for cantaloupe, directly treating fresh-cut pieces of honeydew melon with 50 ppm chlorine or nisin (10 μg/mL)-EDTA (0.02 M) before storage led to lower microbial loads than for water-washed pieces throughout the storage period (Fig. 4).

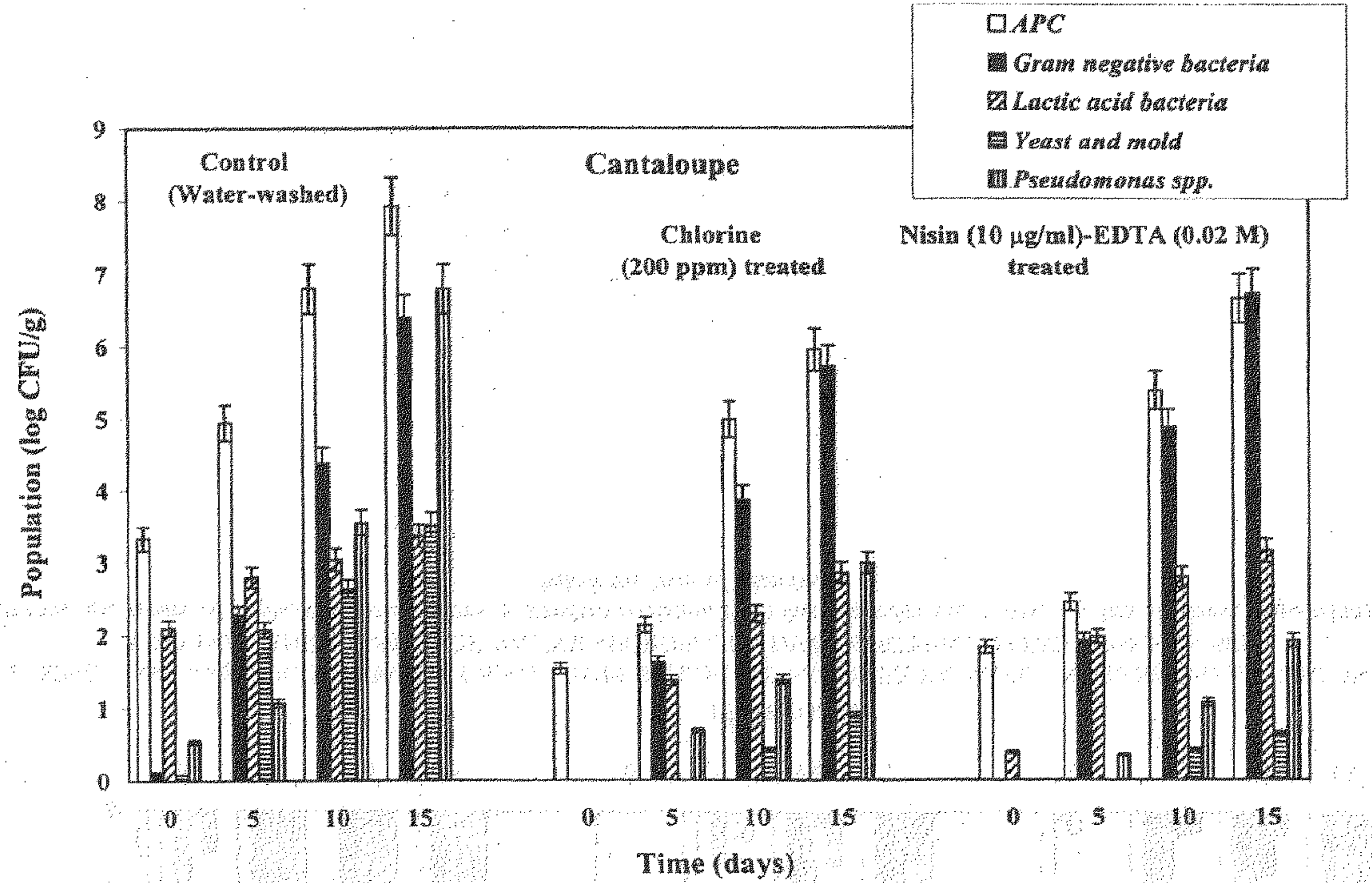

FIG. 1. EFFECT OF WASHING WHOLE CANTALOUPE MELONS ON SURVIVAL AND GROWTH OF NATIVE MICROFLORA ON FRESH-CUT PIECES DURING REFRIGERATED STORAGE (5C)

Values represent means of duplicate determinations ± standard deviation from three experiments. Where no data is shown, populations were below the limit of detection.

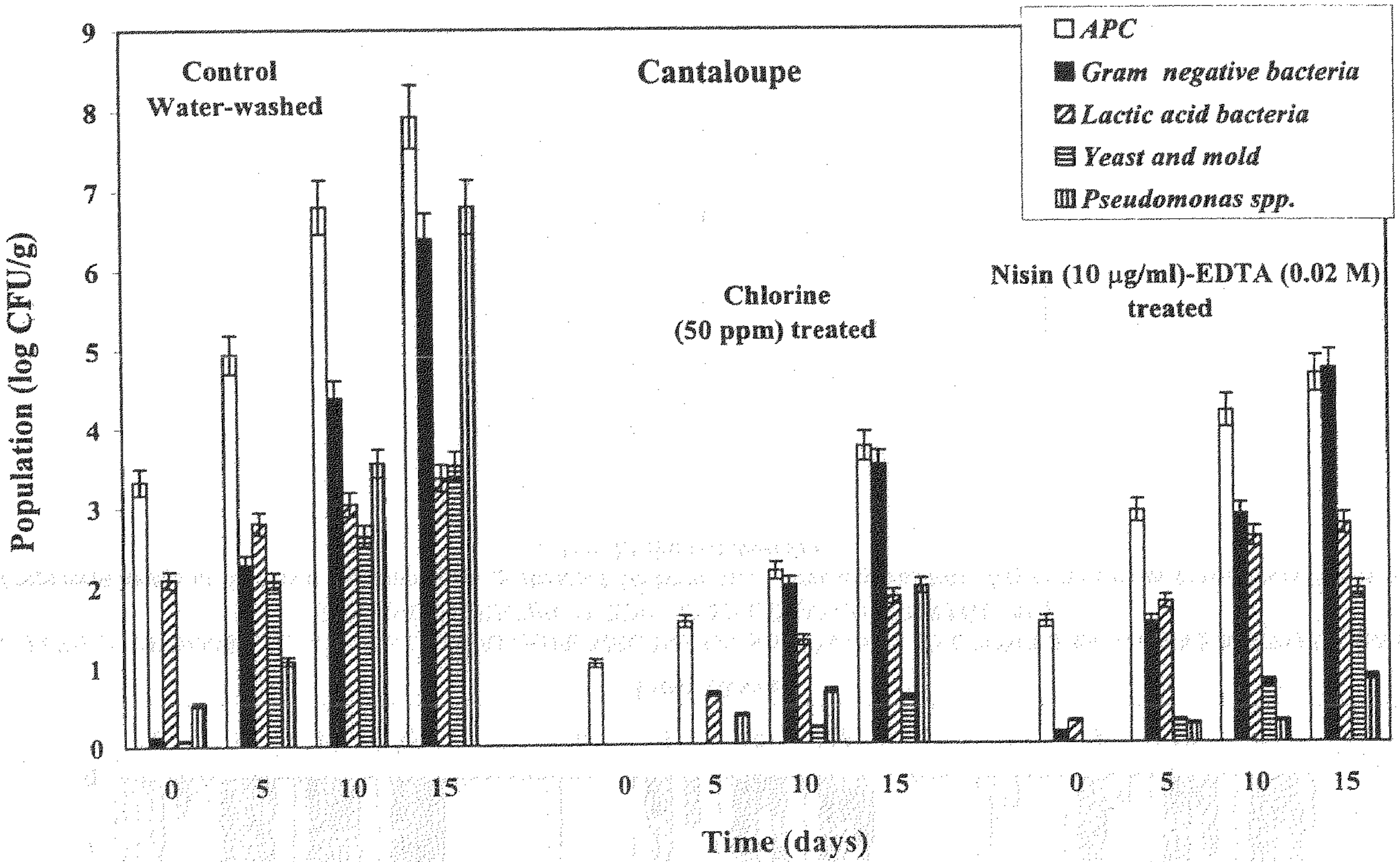

FIG. 2. EFFECT OF WASHING FRESH-CUT CANTALOUPE MELON PREPARED FROM WATER WASHED WHOLE MELONS ON SURVIVAL AND GROWTH OF NATIVE MICROFLORA DURING REFRIGERATED STORAGE (5C)

Values represent means of duplicate determinations ± standard deviation from three experiments. Where no data is shown, populations were below the limit of detection.

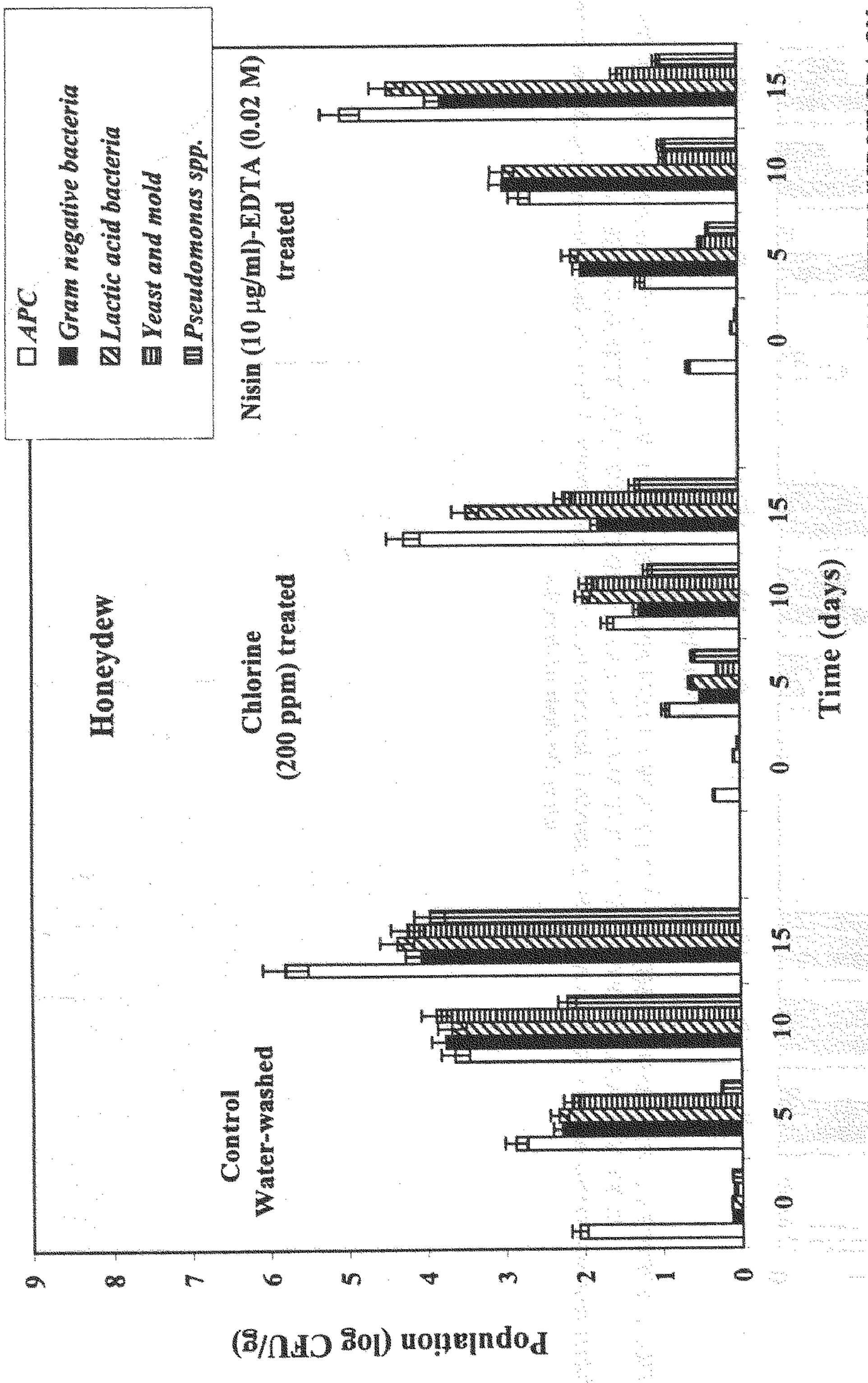

FIG. 3. EFFECT OF WASHING WHOLE HONEYDEW MELONS ON SURVIVAL AND GROWTH OF NATIVE MICROFLORA ON FRESH-CUT PIECES DURING REFRIGERATED STORAGE (5C)

Values represent means of duplicate determinations ± standard deviation from three experiments. Where no data is shown, populations were below the limit of detection.

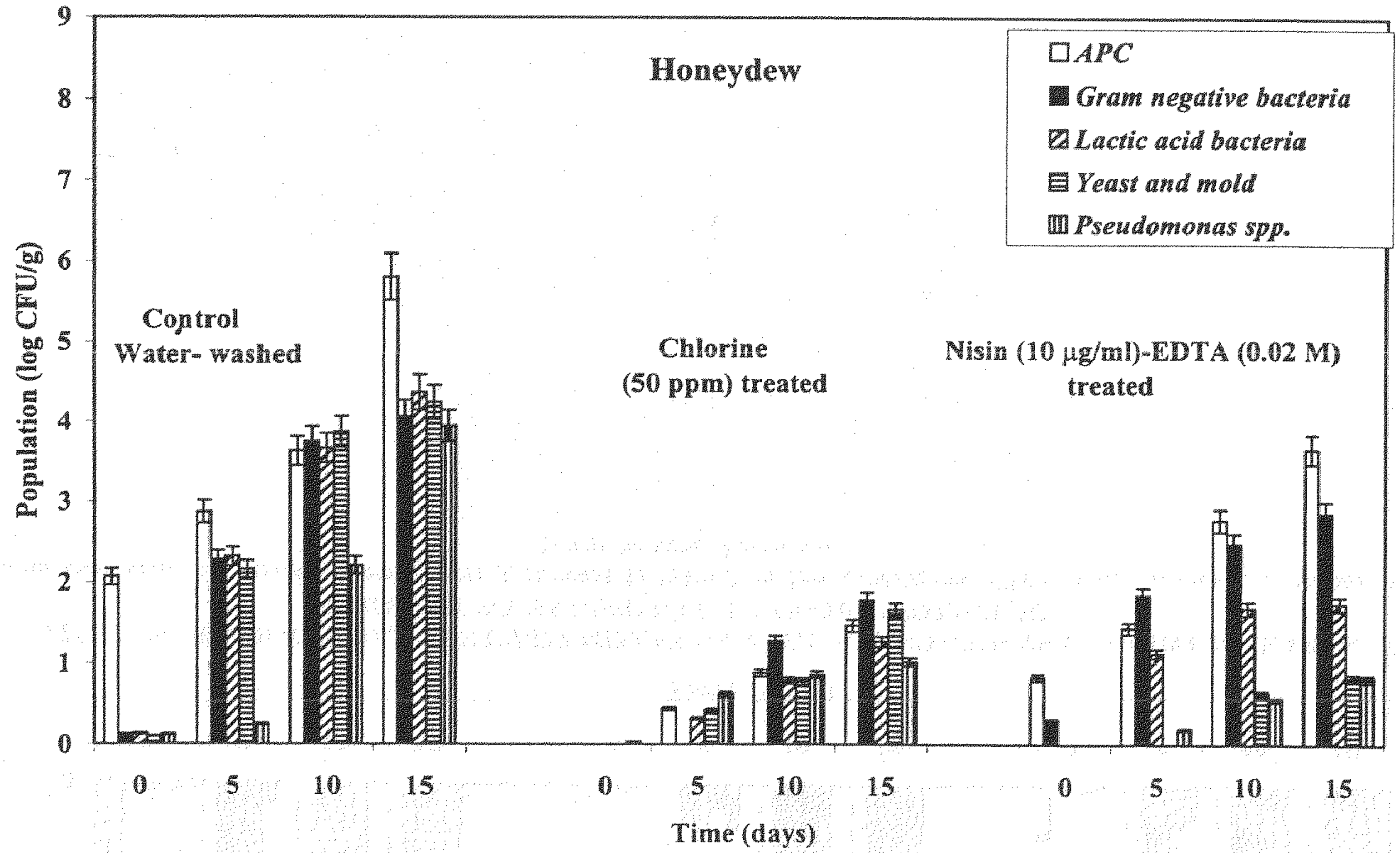

FIG. 4. EFFECT OF WASHING FRESH-CUT HONEYDEW MELON PREPARED FROM WATER WASHED WHOLE MELONS ON SURVIVAL AND GROWTH OF NATIVE MICROFLORA DURING REFRIGERATED STORAGE (5C)

Values represent means of duplicate determinations ± standard deviation from three experiments. Where no data is shown, populations were below the limit of detection.

Effect of Treatment on Quality of Fresh-Cut Melon

Sensory evaluation ratings for changes in appearance, odor and overall acceptability for treated or untreated fresh-cut melon during storage at 5C is shown in Fig. 5, 6 and 7. Appearance ratings for all treated fresh-cut pieces were not significantly different ($P>0.05$) for up to 6 days of storage for cantaloupe and 9 days for honeydew. Significant ($P<0.05$) differences were noted for appearance of fresh-cut cantaloupe prepared from chlorine or nisin-EDTA treated and untreated or water washed whole melon fresh-cut cantaloupes at 9 days compared to 12 days for fresh-cut honeydew pieces. No significant ($P>0.05$) differences in appearance between unwashed and water-washed fresh-cut pieces were found throughout storage. Unwashed fresh-cut pieces became translucent and there was evidence of mold on the surfaces at 12 days of refrigerated storage.

Odor ratings for the fresh-cut pieces during refrigerated storage are shown in Fig. 6. Odor ratings for treated and untreated cantaloupe or honeydew fresh-cut pieces were not significantly different ($P>0.05$) until day 12. At days 12 and 15, differences between chlorine or nisin-EDTA treated and untreated or water washed fresh-cut pieces were significant ($P<0.05$) while differences between nisin-EDTA and chlorine treatments were not significant ($P>0.05$) throughout the storage period. Since differences in odor ratings were noted at days 12 and 15 between treated and untreated fresh-cut pieces, the experiment was discontinued at day 15.

Overall acceptability ratings for fresh-cut pieces were not significantly different ($P>0.05$) between treatments until day 12 of storage where the chlorine or nisin-EDTA treated fresh-cut pieces had much higher overall acceptability ratings (Fig. 7). Ratings for fresh-cut pieces treated with chlorine and nisin-EDTA were not significantly different ($P>0.05$) from each other throughout the storage period.

DISCUSSION

The results of a previous study designed to determine the shelf-life of minimally processed honeydew and cantaloupe melon, kiwifruit, papaya, and pineapple stored at 4C indicated that both the length of shelf-life and type of spoilage were related to the type of fruit (O'Connor-Shaw *et al.* 1994). The authors suggested that the microflora of fruit pieces need to be studied to set appropriate criteria for quality assessment. In our study, we compared five categories of microbes (aerobic mesophilic bacteria, gram-negative bacteria, lactic acid bacteria, yeast and mold and *Pseudomonas* spp.) on the whole

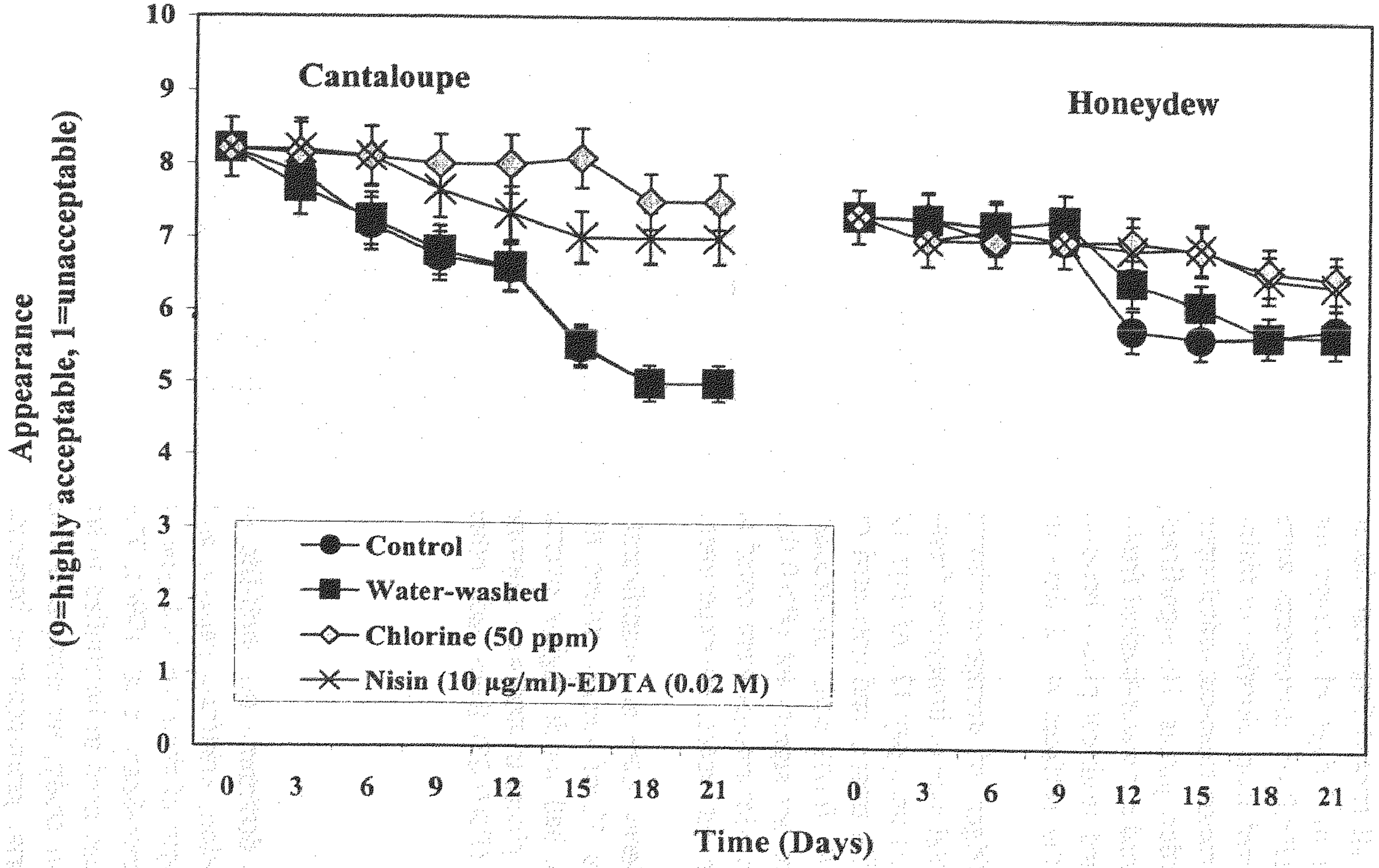

FIG. 5. APPEARANCE RATINGS FOR MINIMALLY PROCESSED FRESH-CUT MELON DURING REFRIGERATED STORAGE (5C)
Values represent means of duplicate determinations ± standard deviation from three experiments.

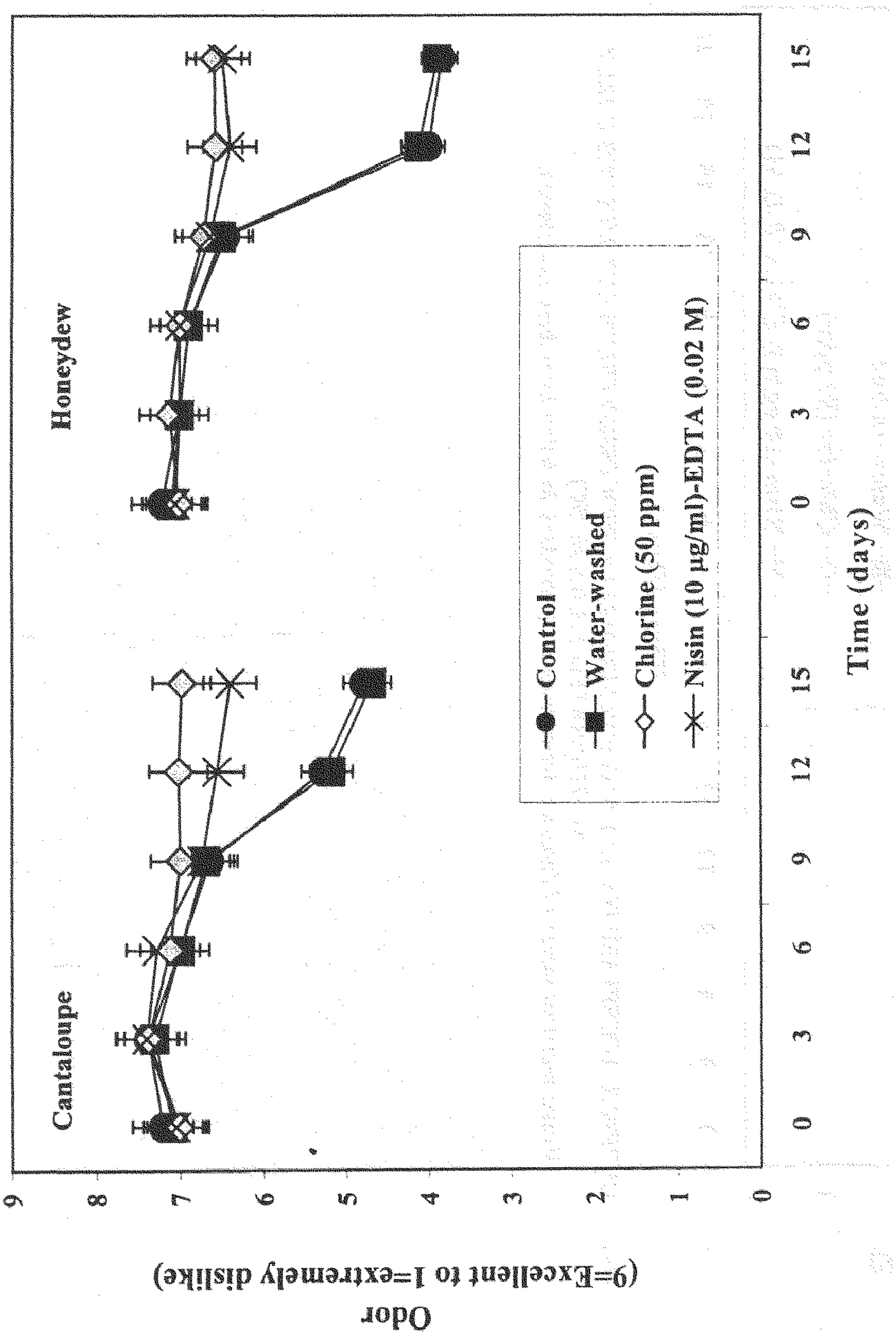

FIG. 6. ODOR RATINGS FOR MINIMALLY PROCESSED FRESH-CUT MELON DURING REFRIGERATED STORAGE (5C)
Values represent means of duplicate determinations ± standard deviation from three experiments.

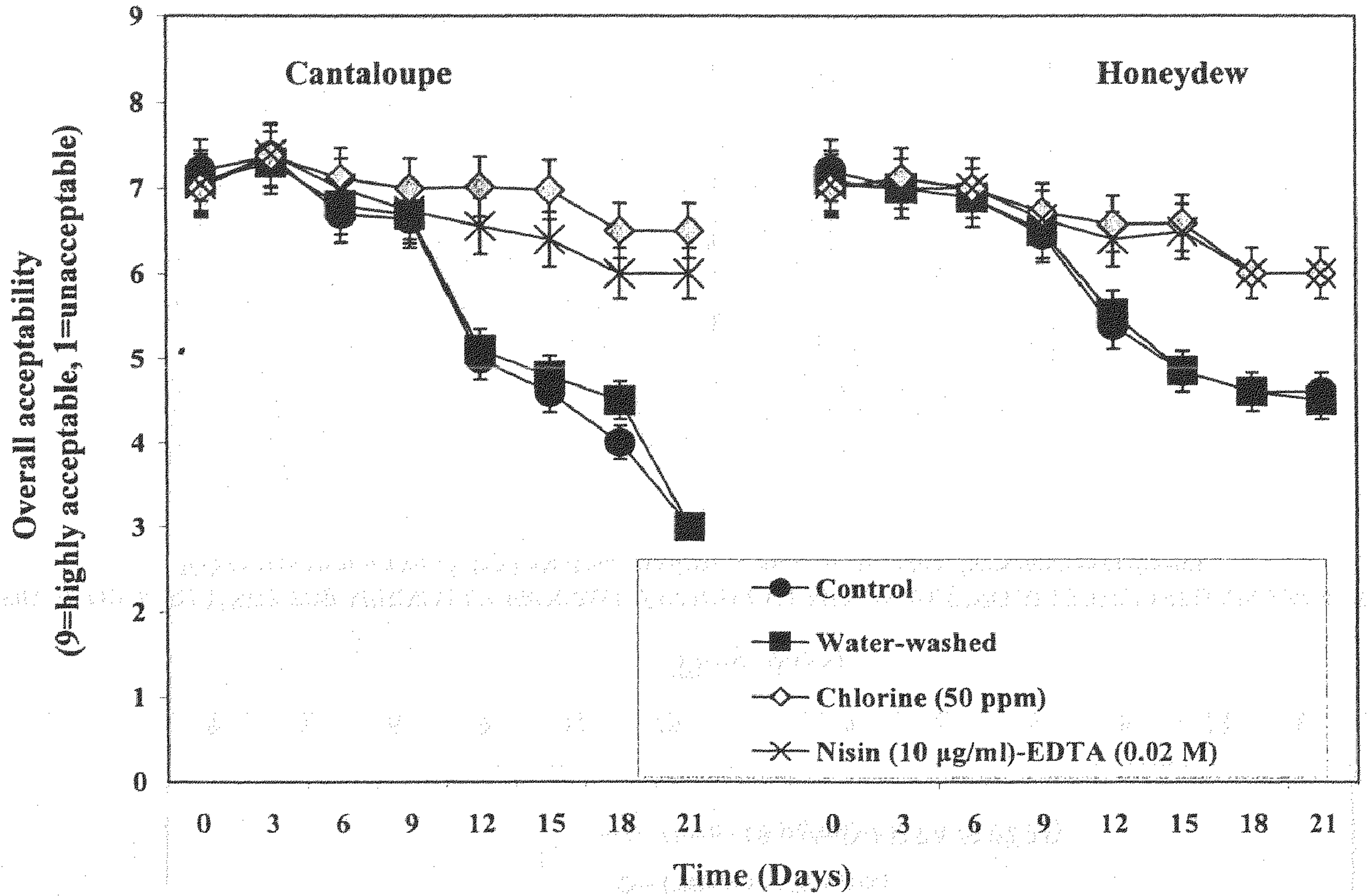

FIG. 7. OVERALL ACCEPTABILITY RATINGS FOR MINIMALLY PROCESSED FRESH-CUT MELONS DURING REFRIGERATED STORAGE (5C)

Values represent means of duplicate determinations ± standard deviation from three experiments.

cantaloupe melon with those of honeydew. The populations for all five categories of microorganisms on cantaloupe were found to be higher than on honeydew melon. The difference in the populations of the native microflora on the honeydew and cantaloupe rind is most likely due to the rough surface of the cantaloupe rind compared to the relatively smooth surface of honeydew melon. The extensive raised netting on the surface of cantaloupe melon no doubt provides more microbial attachment sites and helps to protect attached microbes from being washed from the surface, and possibly from environmental stresses such as UV radiation and desiccation. A previous study yielded microbial counts similar to those found in this study for aerobic mesophiles on unwashed whole cantaloupe and honeydew melons (Ayhan *et al.* 1998). Populations determined in the present study are similar to those previously found in our laboratory (Ukuku *et al.* 2001) for mesophilic aerobes and yeast and mold on cantaloupe melon rind surfaces despite differences in specific culture conditions.

Populations of aerobic mesophilic bacteria on cut vegetables can vary between 10^2 and 10^8 CFU/g with the population size dependent on the type of vegetable (Nguyen and Carlin 2000). As for whole melons, fresh-cut pieces prepared from cantaloupe melon were found to have higher microbial populations than fresh-cut pieces of honeydew throughout storage. This corresponds to the findings of O'Connor-Shaw *et al.* (1994). In unwrapped and wrapped sliced watermelon, *Pseudomonas* spp., *Escherichia coli*, *Enterobacter* spp. and micrococci comprised the predominant microflora (Abbey *et al.* 1988). Fresh-cut vegetables initially harbor lower numbers of microorganisms than do unwashed whole vegetables, as result of washing whole vegetables in chlorinated water before cutting (Li *et al.* 2001). In our study, fresh-cut pieces prepared from whole sanitized melons as well as sanitized fresh-cut pieces harbored lower numbers of microorganisms than did fresh-cut pieces prepared from untreated whole melons and unsanitized fresh-cut pieces. Boyette *et al.* (1993) reported that the microbial decay of minimally processed lettuce is largely due to the growth of microorganisms originating from preharvest environments. Gram-negative bacteria and smaller numbers of yeast make up the bulk of the initial microbial flora of vegetables (Manvell and Ackland 1986; Garg *et al.* 1990; Magnuson *et al.* 1990; King *et al.* 1991; Carlin *et al.* 1995). In stored shredded lettuce, mesophilic aerobic bacteria and psychrotrophic microorganisms tend to predominate, while mold and lactic acid bacteria generally remain low (Garg *et al.* 1990; Barriga *et al.* 1991; Marchetti *et al.* 1992).

The predominant category of microorganisms on fresh-cut cantaloupe was mesophilic aerobic bacteria and lactic acid bacteria immediately after fresh-cut preparation from whole melons washed with water. For fresh-cut honeydew, mesophilic aerobic bacteria predominate immediately after fresh-cut preparation. As days of refrigerated storage increased, the other categories of microbes were detected in all samples irrespective of initial treatments before fresh-cut preparation. The fact that the same categories of microorganisms were detected on whole melon surface and on fresh-cut pieces during storage indicates that the microbes were transferred from the rind to the flesh during fresh-cut preparation.

Washing with water, EDTA or nisin alone did not cause significant reductions of all groups of microorganisms tested for on the outer surfaces of cantaloupe and honeydew melon. However, treatments with a combination of nisin and EDTA did result in significant reductions but overall, were not as effective as the chlorine washes. A synergistic antibacterial activity of nisin and EDTA against soft rotting *Erwinia carotovora*, *E. chrysanthemi*, *Pseudomonas viridiflava* and *P. fluorescens* when cultured in trypticase soy broth has been reported (Wells *et al.* 1998). However, nisin-EDTA treatments were not effective in reducing bacterial soft rot on cut carrot slices. Nisin-chelator treatment of red meat delayed growth of *Staphylococcus aureus* and *Listeria monocytogenes* for a day at room temperature and for up to 2 weeks at 5C (Chung *et al.* 1989). A very slight reduction (0.42 log CFU/cm^2) of *E. coli* O157:H7 and *S. typhimurium* ATCC 14028 attached to meat have been reported for nisin-chelator combinations (Cutter and Siragusa 1995) but not for either alone suggesting inactivity of nisin alone against gram-negative bacteria on meat surfaces. In this study, the combination of nisin-EDTA caused slightly greater log reductions (0.7 to 0.8 log CFU/cm^2) in gram-negative bacteria on whole melon surfaces than for the meat system. This may be attributed to the absence of an endogenous protease enzyme that is associated with meat (Cutter and Siragusa 1995). Although, chlorine or nisin-EDTA combination treatments reduced the native microflora on the surface of whole and fresh-cut melon, elimination of the microflora by these treatments was not achieved. Surface irregularities such as roughness, crevices, and pits have been shown to increase bacterial adherence and reduce the ability of washing treatments to remove bacterial cells (Austin and Bergeron 1995; Frank and Koffi 1990; ICMS 1980). Also, Seo and Frank (1999) reported protection of microorganisms present in cut tissues of lettuce or in stomata from chlorine, which has little penetrating power.

Our results are consistent with reported studies that showed limited bactericidal action (1 to 3 log reductions) of chlorine on produce including whole melons (Ayhan *et al.* 1998; Green and Stumpf 1946; Nguyen and Carlin 1994; Adams *et al.* 1989; Beuchat 1995; Brackett 1992; Ukuku *et al.* 2001). Depending upon the fruit or vegetable and whether it is whole or cut, 200 to 300 ppm chlorine is usually recommended as a sanitizer in wash water (Beuchat 1995). In our study, chlorine (200 ppm) treatment caused approximately 3-log reduction of the total populations of aerobic mesophilic bacteria on whole cantaloupe, and a 2-log reduction on whole honeydew melon. The degree of detachment and/or inactivation of the native microflora by the antimicrobial washing treatments is dependent on the state (example, presence in biofilms) and location of the organisms on the outer surface of the melons.

The visual symptoms of deterioration of fresh-cut produce are flaccidity due to loss of water, changes in color resulting from oxidative browning at the cut surfaces, and microbial contamination (King and Bolin 1989; Varoquaux and Wiley 1994; Brecht 1995). In our study, the washing of fresh-cut pieces in chlorinated water (50 ppm) or nisin-EDTA had a positive effect on the acceptability of the fresh-cut melon presumably by inhibiting microbial growth, resulting in a longer refrigerated shelf-life.

In conclusion, melon rinds harbor a diverse native microflora with higher populations on cantaloupe melon than honeydew melon. Washing treatments using chlorine were more effective in reducing mesophilic aerobic bacteria, gram-negative bacteria and lactic acid bacteria on whole melon surfaces and in minimally processed fresh-cut pieces than nisin-EDTA combination treatments. However, the nisin-EDTA treatment was more effective against yeast and mold and *Pseudomonas* spp. Ratings for odor, appearance, and overall acceptability for fresh-cut pieces treated with chlorine (50 ppm) and nisin-EDTA were improved over water washed pieces after refrigerated storage. No differences between the nisin-EDTA and chlorine treatments in quality ratings were noted. The results of this study suggests that nisin-EDTA can be used to reduce numbers of microbes on whole melon surfaces and also may be used to delay growth of transferred microflora, thus extending the shelf-life of fresh-cut melon. The use of nisin for this application is dependent on regulatory approval. Although nisin-EDTA or chlorine treatments cannot be relied upon to completely sanitize whole cantaloupe or honeydew melons such treatments will reduce the likelihood of contaminated melons reaching the consumer.

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